The Use of Chlorophyll Fluorescence Lifetime to Assess Phytoplankton Physiology within a River-Dominated Environment

C.M. Hall and R.L. Miller, NASA, Earth Science Applications Directorate, Stennis Space Center, MS; *D.G. Redalje*, University of Southern Mississippi, Department of Marine Science, Stennis Space Center, MS; and *S.M. Fernández*, Ciencia, Inc., Hartford, CT

INTRODUCTION

To investigate highly productive regions of river-impacted coastal margins, photosynthetic efficiency along a continuum from rivers into the coastal ocean must be sufficiently analyzed (Cloern, 1987). The Mississippi River plume within the northern Gulf of Mexico is a site ideally suited to study phytoplankton photochemical efficiency within a coastal environment. Previous research has shown that the highest productivity rates and pigment biomass on the Louisiana shelf occur near the edge of the Mississippi River plume within the salinity front created at the river/ocean interface (Lohrenz et al., 1990). Within the plume environment, a low-salinity, riverine layer naturally intrudes into oligotrophic Gulf water (Lohrenz et al., 1990; Lohrenz et al. 1997), and subsequent gradients of salinity, turbidity, and nutrients result from mixing at the plume front. This freshwater flux to the Gulf of Mexico usually establishes two, visually distinct physical zones: "brown," low salinity, turbid waters with high concentrations of inorganic sediments and nutrients and "green" waters with salinities ranging from 20 to 30, increased water clarity, and intermediate concentrations of nutrients. "Blue," high-salinity, offshore water is evident outside the plume area; this region usually contains a well-mixed water column with low nutrient concentrations (Lohrenz et al., 1990; Lohrenz et al., 1997; Redalje et al., 1994).

The application of near real-time fluorescence measurements to natural aquatic ecosystems has provided a basis for understanding short-term acclimations of the photosynthetic apparatus. Measured *in situ*, under ambient irradiance, and without the need for incubation in an enclosed container, variable fluorescence can be used to examine how changes in irradiance and nutrients affect photosynthesis (Falkowski and Kolber, 1993). The quantum yield of fluorescence can be expressed as a ratio between the rate of fluorescence, or the measured lifetime of chl *a* fluorescence, and the total rate of de-excitation, or the natural (intrinsic) lifetime of chl *a* fluorescence. The natural lifetime of the sample (the average time a molecule would spend in the excited state if it were to de-excite by fluorescence only) can be approximated by inoculation of the sample with 3-(3,4-dichlorophenyl)-1, 1-dimethyl urea, or DCMU. The measured lifetime is smaller than the natural lifetime because of the presence of competing de-excitation pathways in photosystem II (PSII) such as photochemistry and heat dissipation. The change in fluorescence lifetime with DCMU inhibition is a measure of the efficiency of photosynthetic electron transport systems (Loftus and Seliger, 1975).

The phase fluorometer is a multi-frequency instrument designed to measure the fluorescence decay of a multi-component sample. Multiple frequencies are required to fully characterize the relative contribution and lifetime of each component within a sample. Lifetime measurement of fluorescence from a heterogeneous sample is made at a single frequency; an apparent phase lifetime is obtained which is a weighted average of the lifetimes of the individual components. When all reaction centers are open, lifetime has a low value and photochemical efficiency is maximal (F_0). When all reaction centers are closed (by the addition of a photosynthetic inhibitor such as DCMU to the sample), lifetime increases and photochemical efficiency is zero (F_m). Thus, the lifetime at any instant is measured, and the photochemical efficiency of this relationship is obtained (Ciencia, 1998).

METHODS

Chlorophyll *a* fluorescence lifetime was measured for phytoplankton populations inhabiting the three physical zones surrounding the Mississippi River's terminus in the Gulf of Mexico. Observations of river discharge volume, nitrate + nitrite, silicate, phosphate, PAR (Photosynthetically Active Radiation) diffuse attenuation within the water column, salinity, temperature, SPM, and chl *a* concentration were used to characterize the distribution of chl fluorescence lifetime in a given region within restricted periods of time. Thirty-three stations extending from the Mississippi River plume to the shelf break of the Louisiana coast were surveyed for analysis of chlorophyll fluorescence lifetime during two cruises conducted March 31 – April 6, 2000, and October 24 – November 1, 2000. At each station, two to three depths were chosen for fluorescence lifetime measurement to represent the vertical characteristics of the water column. Where possible, samples were taken from just below the surface and from just above and below the pycnocline. All samples collected were within the 1% light level of the water column (the euphotic zone). Upon collection, samples were transferred to amber Nalgene bottles and left in the dark for at least 15 minutes to reduce the effects of non-photochemical quenching and to insure that photosynthetic reaction centers were open. Before measurements within the phase fluorometer were begun, the instrument was allowed to warm up for no less than one hour.

Measurement of fluorescence lifetime involved three steps suggested by the manufacturer of the instrument (Ciencia, 1998). First, samples were drawn from the amber Nalgene bottles through a Pasteur pipette into the round, polished cuvette that fits into the sample compartment of the phase fluorometer. This first measurement establishes the strength of the fluorescence emission from the sample and allows for the instrument gain within the phase fluorometer to be adjusted automatically. The phase fluorometer includes an optical variable attenuator in the emission path and a software-controlled (Labview®) variable gain amplifier. These two items control the overall gain of the instrument. Second, after this initial sample is removed and discarded, a scattering sample is withdrawn from a glycogen solution by Pasteur pipette into the sample cuvette. The glycogen solution consists of 0.01 ± 0.005 g oyster glycogen powder in 250mL Milli-Q water. During measurement of the scattering sample, the optical variable attenuator within the phase fluorometer is adjusted to bring the detected signal from the scatterer to the same amplitude as that of the fluorescent sample used in the previous step. The phase delay corresponding to the scattering sample is then measured and stored by the instrument software. The third step in fluorescence lifetime measurement involves drawing a new sample from the Nalgene bottle and replacing the scattering sample with the new fluorescent sample. Since the samples under examination are photochemically active, the sample used in the gain-setting step should not be reintroduced into the instrument. However, the two samples should be aliquots from the same source. The phase fluorometer then measures the phase delay due to the second fluorescent sample. By using the stored value of the phase delay from the scattering sample, the phase fluorometer calculates a measured fluorescence lifetime (τ). The three steps are repeated for new fluorescent samples from the same aliquot but with several drops of highly concentrated DCMU added to each fluorescent sample. The lifetime calculated from the DCMU-treated samples is the natural, or intrinsic, fluorescence lifetime (τ_{DCMU}).

The quantum yield of fluorescence, as estimated by the phase fluorometer, can be calculated from the ratio of the measured lifetime (τ) and the natural lifetime (τ_{DCMU}) of a sample:

$$\phi_f = k_f/(k_f + k_p + k_d + k_t) = \tau/\tau_{DCMU},$$

where the quantum yield of fluorescence is equal to the ratio of the first-order rate constant of fluorescence (k_f) and the sum of the first-order rate constants of trapping at the reaction center or photochemical quenching (k_p), nonradiative deactivation (k_d), and transfer to another species (k_t) (Loftus

and Seliger, 1975; Ciencia, 1999). Thus, a change in the quantum yield of fluorescence denotes a change in fluorescence lifetime. Furthermore, the lifetimes of samples before and after DCMU addition can be used to obtain an estimate of variable fluorescence:

$$(\tau_{DCMU} - \tau)/ \ \tau_{DCMU} = (F_m - F_o)/ \ F_m = F_v / \ F_m,$$

where F_0 is the measured lifetime of the sample, F_m is the maximum fluorescence obtained by DCMU addition to the sample, and variable fluorescence (F_v) is estimated by the difference in lifetime before and after DCMU addition. Measurements of variable fluorescence are based on the Kautsky effect, the kinetics of which result from the time-dependent quenching of fluorescence (Kautsky and Hirsch, 1931).

RESULTS

Measured fluorescence lifetime (τ_0) was not statistically different between cruises, but intrinsic lifetime (τ_{DCMU}), fluorescence quantum yield (ϕ_f), and F_v/F_m displayed significant differences between cruises. During the spring cruise, τ_0, ϕ_f, and F_v/F_m were significantly different between brown, green, and blue samples. None of these parameters was different between water types sampled during the fall cruise. Mean values of τ_{DCMU} ranged from 1.34 ± 0.240 ns in spring 2000 to 1.53 ± 0.193 ns in fall (Figure 1). Fluorescence quantum yield and F_v/F_m ranged from 0.649 ± 0.171 and 0.351 ± 0.171, respectively, in spring to 0.612 ± 0.188 and 0.388 ± 0.188 in fall (Figure 1). During the spring cruise, ϕ_f was highest in blue water (0.734 ± 0.236) and F_v/F_m was highest in green water (0.394 ± 0.136) (Figure 1). For both cruises, sampling of fluorescence parameters at discrete depths indicated lower values at the base of the euphotic zone when compared to values measured at the surface. Differences between surface values and values measured at the base of the euphotic zone were more pronounced in blue water when compared to values in brown and green water.
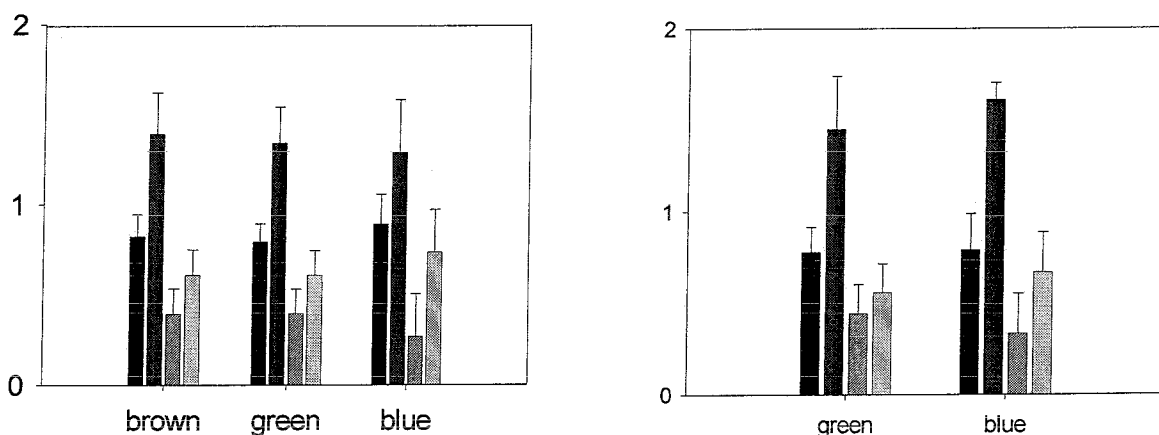

Figure 1. Mean measured lifetime (black), natural lifetime (red), F_v/F_m (green), and quantum yield of fluorescence (yellow) measured during spring 2000 (left) and fall 2000 (right) within brown, green, and blue water types. Due to extremely low river discharge, brown water was not encountered during the fall 2000 cruise

The highest maximum value of F_v/F_m was encountered in blue water (0.65) during the spring cruise (Figure 2) and in green water during the fall cruise (0.63) (Figure 3), although the mean value in blue water in spring was much lower than the mean green-water value during the same cruise. When values of F_v/F_m were combined for all cruises and subjected to a one-sample t-test with an hypothesized mean of 0.65, mean F_v/F_m within all water types differed significantly from the hypothesized mean. Furthermore, mean F_v/F_m in each water type overlapped. Values of F_v/F_m did not approach the maximum value of 0.65 in green water and were not significantly lower in brown and blue water (Figure 2).
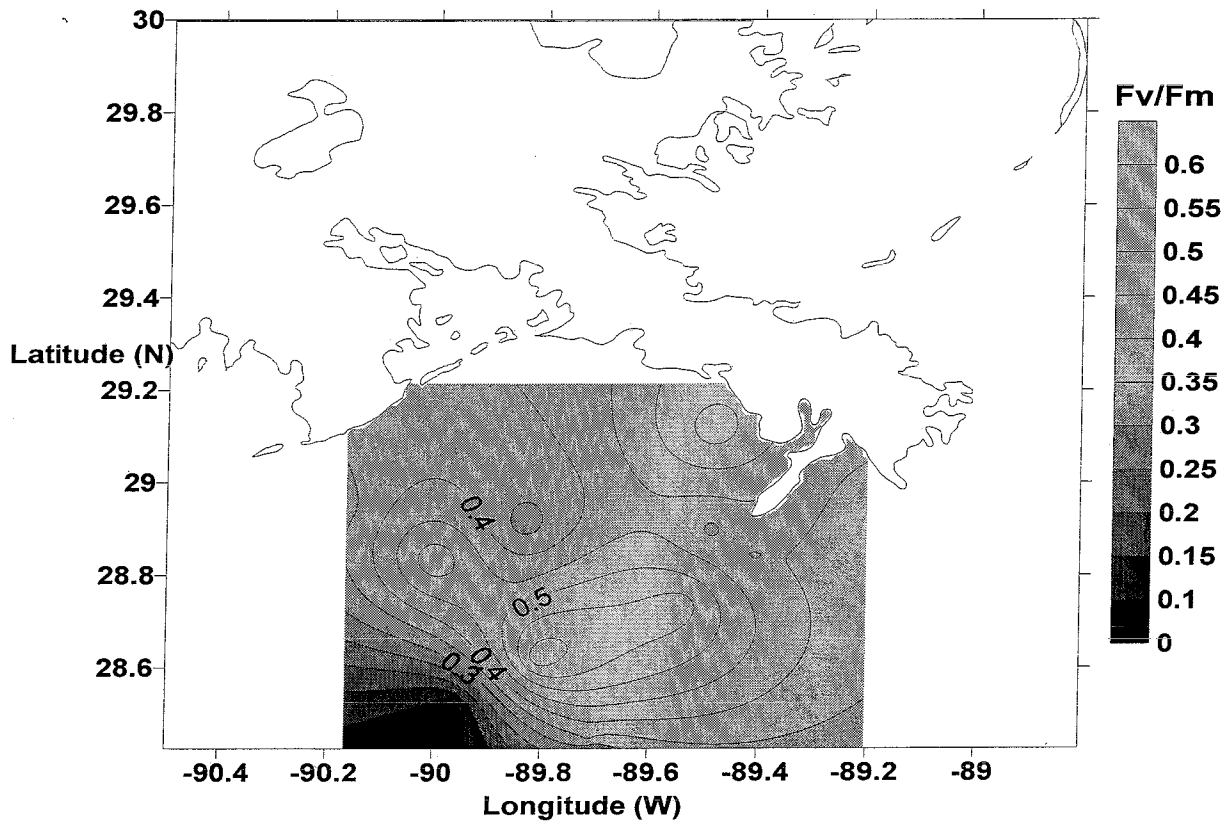

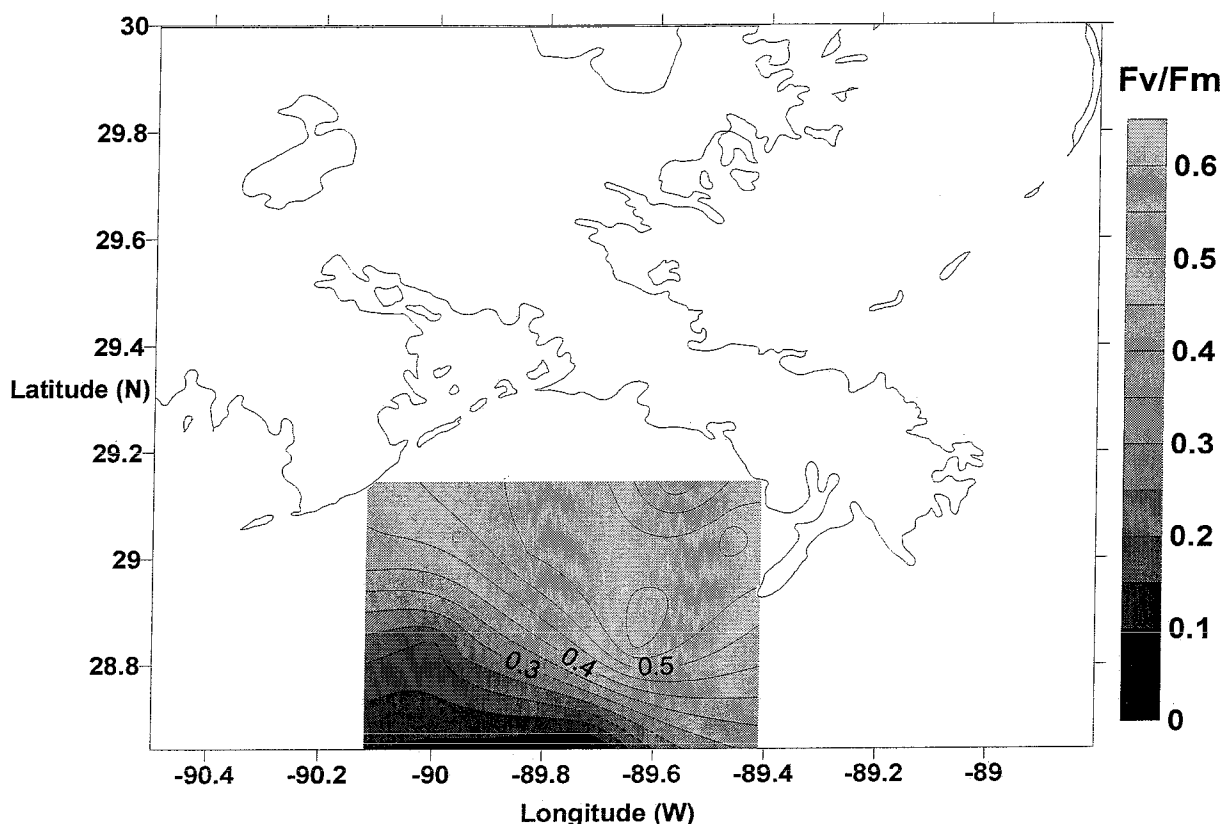*Figure 2. Contour of F_v/F_m measured during spring 2000 cruise.*

Figure 3. Contour of F_v/F_m measured during fall 2000 cruise.

DISCUSSION

In phytoplankton cultures, F_v/F_m has been observed to reach an absolute maximum of 0.65 under nutrient replete conditions. Only in nutrient-rich estuaries have similarly high values been observed in nature (Olaizola *et al.*, 1996). Values of F_v/F_m approaching 0.60 have been reported for coastal waters (Kolber *et al.*, 1990; Geider *et al.*, 1993; Olaizola *et al.*, 1996), but F_v/F_m rarely exceeds 0.5 in offshore surface waters (Falkowski *et al.*, 1992; Geider *et al.*, 1993; Greene *et al.*, 1994; Kolber and Falkowski, 1993; Olaizola *et al.*, 1996). Within the Mississippi River plume, F_v/F_m ranged from 0.11 to 0.61 in spring 2000 and from 0.13 to 0.63 in fall 2000. During both seasons, the majority of F_v/F_m values in the plume (brown and green waters) were between 0.30 and 0.49. In spring 2000, 16% of all F_v/F_m measurements from offshore waters were greater than 0.60, while only 9% and 4% of all measurements from brown and green waters were greater than 0.60, respectively. Conversely, in October, no F_v/F_m values from blue water were greater than 0.60, and only 8% of all measurements from green water were greater than 0.60. Although high F_v/F_m was encountered in blue water, 42% of all F_v/F_m measurements (recorded for both sampling times) in this water type were less than 0.20. Based on previous research,

one would expect higher F_v/F_m than what was measured for an environment receiving constant input of inorganic nutrients. The values encountered during both cruises are indicative of populations experiencing nutrient limitation (Olaizola et al., 1996). Areas of potential nutrient limitation, based on the index of Dortch and Whitledge (1992) were encountered within the plume during both cruises. Phosphate concentrations were in limiting concentrations during the spring cruise, and nitrate concentrations were in limiting concentrations during the fall cruise. During the spring cruise, only one offshore station displayed potentially limiting nutrient concentrations (nitrogen-limited) compared to 75% of all brown water samples (phosphorus-limited). During the fall cruise, NO_x and PO_4 exhibited potentially limiting concentrations at several green and blue stations.

Further investigation is needed to explore the potential of fluorescence lifetime measurements in characterization of phytoplankton physiology within the Mississippi River plume environment. Several cruises throughout the year, spanning seasonal differences in river discharge, would aid in the assessment of phytoplankton photosynthetic efficiency along an estuarine continuum. However, this research provides a basis for further study in the use of measurements based on chl fluorescence lifetime to understand phytoplankton dynamics within a physically variable environment.

REFERENCES

Ciencia, Inc. (1998). Operating instructions: fluorescence lifetime phytoplankton analyzer. Prototype 4.0. Contract NAS13-97033. 22 pp.

Ciencia, Inc. (1999). Ground truth phytoplankton sensor. NAS13-97033. Performing organization report 121202-FR. 23 pp.

Cloern, J.E. (1987). Turbidity as a control on phytoplankton biomass and productivity in estuaries. Continental Shelf Research 7 (11/12): 1367-1381.

Dortch, Q. and T.E. Whitledge. (1992). Does nitrogen or silicon limit phytoplankton production in the Mississippi River plume and nearby region? Continental Shelf Research 12: 1293-1309.

Falkowski, P.G., Greene, R.M., and R.J. Geider. (1992). Physiological limitations on phytoplankton productivity in the ocean. Oceanography 5 (2): 84-91.

Geider, R.J., Greene, R.M., Kolber, Z., MacIntyre, H.L., and P.G. Falkowski. (1993). Fluorescence assessment of the maximum quantum efficiency of photosynthesis in the western North Atlantic. Deep-Sea Research I 40(6): 1205-1224.

Greene, R.M., Kolber, Z., Swift, D.G., Tindale, N.W., and P.G. Falkowski. (1994). Physiological limitation of photosynthesis in the eastern equatorial Pacific determined from variability in the quantum yield of fluorescence. Limnology and Oceanography 39(5): 1061- 1074.

Kautsky, H. and A. Hirsch. (1931). Neue Versuche zur Kohlensaureassimilation. Naturwissenschaften 19: 964.

Kolber, Z., Wyman, K.D., and P.G. Falkowski. (1990). Natural variability in photosynthetic energy conversion efficiency: a field study in the Gulf of Maine. Limnology and Oceanography 35(1): 72-79.

Kolber, Z. and P.G. Falkowski. (1993). Use of active fluorescence to estimate phytoplankton photosynthesis in situ. Limnology and Oceanography 38(8): 1646-1665.

Loftus, M.E. and H.H. Seliger. (1975). Some limitations of the in vivo fluorescence technique. Chesapeake Science 16 (2): 79-92.

Lohrenz, S.E., Dagg, M.J., and T.E. Whitledge. (1990). Enhanced primary production at the plume/oceanic interface of the Mississippi River. Continental Shelf Research 10 (7): 639-664.

Lohrenz, S.E., Fahnenstiel, G.L., Redalje, D.G., Lang, G.A., Chen, X., and M.J. Dagg. (1997). Variations

in primary production of northern Gulf of Mexico continental shelf waters linked to nutrient inputs from the Mississippi River. *Marine Ecology – Progress Series* 155: 45-54.

Olaizola, M., Geider, R.J., Harrison, W.G., Graziano, L.M., Ferrari, G.M., and P.M. Schlittenhardt. (1996). Synoptic study of variations in the fluorescence-based maximum quantum efficiency of photosynthesis across the North Atlantic Ocean. *Limnology and Oceanography* 41(4): 755-765.

Redalje, D.G., Lohrenz, S.E., and G.L. Fahnenstiel. (1994). The relationship between primary production and the vertical export of particulate organic matter in a river-impacted coastal ecosystem. *Estuaries* 17 (4): 829-838.

REPORT DOCUMENTATION PAGE

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
01-05-2002		

4. TITLE AND SUBTITLE
The Use of Chlorophyll Fluorescence Lifteme to Assess Phytoplankton Physiology within a River-Dominated Environment

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Callie M. Hall
Richard L. Miller
Donald G. Redalje
Salvador M. Fernandez

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
SSC Earth Science Applicaitons Directorate

8. PERFORMING ORGANIZATION REPORT NUMBER

SE-2002-03-00018-SSC

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITOR'S ACRONYM(S)

11. SPONSORING/MONITORING REPORT NUMBER

12. DISTRIBUTION/AVAILABILITY STATEMENT
Publicly Availability STI per form 1676

13. SUPPLEMENTARY NOTES
Conference - Seventh Internaitonal Conference of Remote Sensing for Marine and Coastal Environments, Miami FL

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:

a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19b. NAME OF RESPONSIBLE PERSON
U		U	UU	7	Callie Hall

19b. TELEPHONE NUMBER (Include area code)
(228) 688-2360